Progress Report on Funded Nursery Projects
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Project Title: Seed transmission of *Cherry leafroll nepovirus.*

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FINAL REPORT:

Cherry leafroll nepovirus is believed to be a relatively new introduction into the cherry production areas of the Pacific Northwest. Anecdotal evidence from affected grower operations suggests that the virus has only been causing significant disease in the area for approximately 12 years. This virus causes a debilitating decline of cherry trees when it occurs in the presence of either *Prunus necrotic ringspot virus* or *Prune dwarf virus*. The latter two viruses are endemic in all cherry growing areas of the world, and begin to encroach in most orchards when the trees are 10 to 12 years old. Thus, many cherry trees infected with *Cherry leafroll virus* begin a rapid and severe decline at a time when production should be at its maximum. Current estimates suggest that there are relatively few trees infected with *Cherry leafroll virus* in the industry. Unfortunately, they are scattered through much of the cherry production area. We must quickly learn what precautions are needed to keep infected trees from entering the nursery trade.

Most members of the genus *Nepovirus* are transmitted by pollen, or nematodes, or both. Our data has indicated that pollen is a rich source of this virus. This raises the specter that virus-infected seedling rootstock could be produced from seed that developed on otherwise healthy trees if virus-contaminated pollen is present. Our research addressed this risk as a possible route by which *Cherry leafroll virus* could enter the nursery trade.

We evaluated the seed transmission of *Cherry leafroll virus* in four commercial blocks with a known presence of this virus. Trees that were sampled were either 'Bing', 'Van' or mazzard seedlings with the adjacent trees being a mixture of 'Bing', 'Van' and mazzard. Virus status of all trees was determined by serological testing of young flower buds collected at popcorn stage. Samples were collected from all scaffold limbs and tested for *Cherry leafroll virus.* Because of the interaction between *Cherry leafroll virus* with *Prunus necrotic ringspot virus* and *Prune dwarf virus* in terms of symptoms development, the possibility exists that mixed infections could affect embryo viability. Therefore, all samples were tested for the latter two viruses as well.

Where the maternal (seed bearing) trees are positive for *Cherry leafroll virus,* a high percentage of pits from each of two trees contained viral antigen (ranging from 74% to 90%). The remaining seed collected from these sources was allowed to germinate and a significant percentage of the resulting seedlings were also infected with *Cherry leafroll virus* (17%). Mixed infections of *Cherry leafroll virus, Prunus necrotic ringspot virus* and *Prune dwarf virus* were evident in some seedlings, thus embryos supporting mixed infections were viable.

Where the maternal trees were negative for *Cherry leafroll virus* but adjacent to virus-infected pollen sources, the percentage of pits containing detectable antigen varied from 0 to 23%. In the first year trial, ambiguities arose because a tree that was used as seed source (treatment I, tree R10T3) and tested negative for *Cherry leafroll virus* became infected during the course of the study. Thus, some of the spurs may have already been infected with *Cherry leafroll virus* at the time of fruit sampling. To clarify the importance of virus status of the seed-bearing trees in the

second year of trials, fruit flesh was also tested for the presence of virus. This has proven to be a reliable indicator of the virus status of the spur from which the fruit originated. None of the fruit from the virus-negative source trees in the repeated experiment were positive for *Cherry leafroll virus*, thus insuring an accurate interpretation of results. However, as in the first year of trials, virus antigen was detected in a low percentage of fruit pits from these trees, but none of the resulting seedlings were infected with *Cherry leafroll virus.*

Table 1. Sweet cherry trees in commercial orchards were tested for Cherry leafroll virus (CLRV), Prunus necrotic ringspot virus (PNRSV) and Prune dwarf virus (PDV). Fruit was harvested from these trees. The endocarp was collected from a sub-set of pits and tested serologically for the presence of the same viruses. The remaining seeds were stratified and allowed to germinate. Seedlings were tested at the four-leaf stage.

Description of trees from which fruit was collected and tested.	**Viruses detected**		
	CLRV	**PNRSV**	**PDV**
Treatment I: CLRV-free maternal tree adjacent to CLRV-infected pollinators			
Tree R8T5, 'Van' (year 1):	-	-	+
virus status of adjacent trees (3 missing), n=6	2/6	0/6	2/6
pits ; n=100	0%	0%	88%
seedlings; n=333	**0%**	**1%**	**30%**
Tree R10T3, 'Van' (year 1):	-	-	+
virus status of adjacent trees (1 missing)' n=7	3/7	0/7	4/7
pits; n=100	23%	0%	58%
seedlings; n=282	**0%**	**0%**	**9%**
Tree R6T20, 'Bing' (year 2):	-	-	+
virus status of adjacent trees, n=8	2/8	0/8	2/8
fruit; n=50	0%	0%	4%
pits ; n=50	0%	0%	86%
seedlings; n=3	**0%**	**0%**	**0%**
Tree R6T21, 'Bing' (year 2):	-	-	+
virus status of adjacent trees, n=8	1/8	0/8	2/8
fruit; n=50	0%	0%	18%
pits; n=50	6%	0%	90%
seedlings; n=0 (no germination)	**NT**[1]	**NT**	**NT**
Tree R26T12, 'Bing' (year 2):	-	-	-
virus status of adjacent trees, n=8	3/8	0/8	0/8
fruit; n=50	0%	0%	0%
pits; n=50	1%	0%	0%
seedling; n=31	**0%**	**0%**	**0%**
Tree R27T13, 'Bing' (year 2):	-	-	-
virus status of adjacent trees (4 missing); n=4	1/4	0/4	0/4
fruit; n=50	0%	0%	0%
pits; n=50	0%	0%	0%
seedlings; n=10	**0%**	**0%**	**0%**
TOTAL SEEDLINGS; n=659	**0%**	-	-

Table I cont'd...

Treatment II: CLRV-infected maternal tree			
Tree R13T3, mazzard seedling (year 1):	+	+	+
virus status of adjacent trees; n=6	1/6	5/6	5/6
pits; n=100	**76%**	**60%**	**96%**
seedlings; n=228	**21%**	**29%**	**18%**
Tree R13T5, 'Bing' (year 1):	+	-	+
virus status of adjacent trees (1 missing); n=5	1/5	4/5	4/5
pits; n=100	**74%**	**0%**	**98%**
seedlings; n=70	**4%**	**0%**	**31%**
Tree R5T19, 'Bing' (year 2):	+	-	-
virus status of adjacent trees ; n=8	3/8	0/8	1/8
fruit; n=30	**100%**	**0%**	**0%**
pits; n=30	**90%**	**0%**	**0%**
seedlings; n=9	**11%**	**11%**	**0%**
TOTAL SEEDLINGS; n=307	**17%**	-	-
Treatment III: CLRV-free maternal tree and adjacent pollinators			
Tree R7T5, 'Bing' (year 1): ~56 ft from nearest CLRV infected pollinator	-	±	+
virus status of adjacent trees (3 missing); n=5	0/5	2/5	5/5
pits; n=100	**0%**	**2%**	**100%**
seedlings	**NT**	**NT**	**NT**
Tree R13T24, 'Bing' (year 1): ~200 ft from nearest CLRV infected pollinator	-	-	+
virus status of adjacent trees; n=6	0/6	NT	NT
pits; n=50	**0%**	**0%**	**98%**
seedlings	**NT**	**NT**	**NT**

1. NT= Not tested.

In a related study funded by the Washington Tree Fruit Research Commission, we are exploring the role of pollen in the transmission of *Cherry leafroll virus* through other routes. During the course of that study, approximately 6000 fruits were harvested from a 'Bing' tree that tested negative for *Cherry leafroll virus* and located adjacent to a 'Van' pollinator tree that tested positive and expressed severe symptoms of *Cherry leafroll virus*. Initially, 200 fruits were analyzed by ELISA (table 2). Subsequently, the pits alone were tested from another 2600 fruit. In the same block, the fruit from a 'Van' tree adjacent to a virus-infected 'Bing' was analyzed. The results from this study substantially reflect the results from the WSDA research trials. However, the larger number of harvested fruit will allow us to test a larger population of seedlings during the summer of 2005. This latter aspect of the research will be performed with funds from other sources, but the data will be blended into the current information for reporting purposes.

Table 2: Fruit was harvested from a tree that had previously tested negative for *Cherry leafroll virus*, but was situated adjacent to an infected tree that could act as pollinator. Fruit flesh, pits and pedicels were tested separately by ELISA

Tree identification	**ELISA results** number positive/number tested (percentage positive)		
	Fruit	Pits	Pedicel
R1T5 'Bing'	0/200 (0.0%)	45/200 (22.5%)	0/200 (0.0%)
R1T5 'Bing'	---	334/2600 (12.8%)	---
R15T10 'Van'	0/400 (0.0%)	7/400 (1.8%)	0/400 (0.0%)

CONCLUSIONS:

The association of *Cherry leafroll virus* with pollen poses a concern for the production of cherry trees in the nursery. In terms of the production of seedling rootstock, it appears from the accumulated data that if the mother trees are free of *Cherry leafroll virus*, then the risk of virus infected seedlings is very low. None of the 659 seedlings tested in this trial were infected with *Cherry leafroll virus*, despite the detection of virus antigen by ELISA in 37 (9%) pits of the 400 tested. However, the importance of insuring that the seed-bearing trees remain free of *Cherry leafroll virus* is equally evident. When the maternal tree is infected, 52 (17%) of the 307 resulting seedlings are infected with *Cherry leafroll virus*.

Thus, nursery production of seedling rootstock is relatively safe from the infrequent and sporadic introduction of virus-laden pollen. However, periodic monitoring of seed source trees is a prudent preventative measure to reduce the inadvertent production of virus-infected seedling rootstocks.